AD_____

Award Number: DAMD17-00-1-0282

TITLE: Semi-Synthesis and In-Vitro Anticancer Evaluation of Derivatives of a New Microtubule Poison with a Taxol-Like Mechanism

PRINCIPAL INVESTIGATOR: Thomas Hemscheidt, Ph.D.

CONTRACTING ORGANIZATION: University of Hawaii
Honolulu, Hawaii 96822

REPORT DATE: September 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2002	Annual (1 Sep 01 - 31 Aug 02)

4. TITLE AND SUBTITLE
Semi-Synthesis and In-Vitro Anticancer Evaluation of Derivatives of a New Microtubule Poison with a Taxol-Like Mechanism

5. FUNDING NUMBERS
DAMD17-00-1-0282

6. AUTHOR(S):
Thomas Hemscheidt, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Hawaii
Honolulu, Hawaii 96822

E-Mail:tomh@gold.chem.hawaii.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract *(Maximum 200 Words)* ***(abstract should contain no proprietary or confidential information)***

In prior research we had discovered a steroid-like molecule with antimicrotubule activity of the Taxol-like mechanism of action from a tropical ornamental plant. There are only a few molecules known that have this type of activity. One of these is clinically used, the second one, the epothilones, is under development. The aim of the present award is to modify the structure of the compound we have discovered in order to understand which features of the molecule are important for biological activity. Five derivatives have been prepared, which will be evaluated in mechanism-based and in cytotoxicity assays.

14. SUBJECT TERMS
drug discovery, antimicrotubule agents, semisynthesis

15. NUMBER OF PAGES
8

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction

Under prior funding we had discovered a new microtubule agent with a taxol-like mechanism of microtubule stabilization from a tropical ornamental plant, *Tacca chantrieri*. The compound is a highly oxygenated steroid-like material in which the carbon atoms of the aliphatic side chain of cholesterol are oxidatively cyclized onto the D-ring of the basic steroid skeleton. The resulting compound has six rings, which are further modified through oxidation and acetylation.

The high degree of oxygenation gives the material a substantial polarity, which is not desirable in a drug-like molecule. Moreover, the oxygenation introduces a large number of chiral centers, which in turn make taccalonolide an extremely challenging target for synthesis.

The purpose of the present research is to modify the structure of taccalonolide A in such a way that the importance of these functional groups on the biological activity, i.e. the antimicrotubule activity and cyotoxicity can be assessed. Two strategies can be pursued to this end, one semisynthetic, i.e. through functional group modification, and one synthetic, i.e. by introducing complexity into simple starting materials. Under this award the former strategy is being pursued. It is hoped that at the end of this research it will be possible to propose a simplified structure, which not only retains biological activity or even shows improved potency, but also is synthetically accessible.

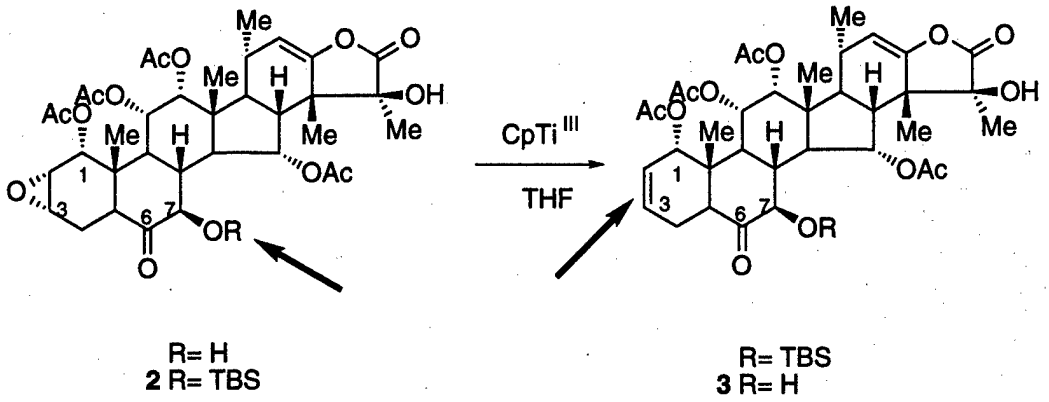
During our earlier isolation studies we did not find any other taccalonolide-related, which already had structural differences and different biological activities. Such observations might have given us some indications as to where to start with our modifications. In the absence of this type of information we have to systematically go around the structure with the aim of deleting functionality in a systematic way and probing the influence on the biological activity. Initial efforts have concentrated on reactive sites such as the epoxide function, the ketone and the enolester.

BODY

Task 3

<u>Improved preparation of the desepoxy derivative of taccalonolide A</u>

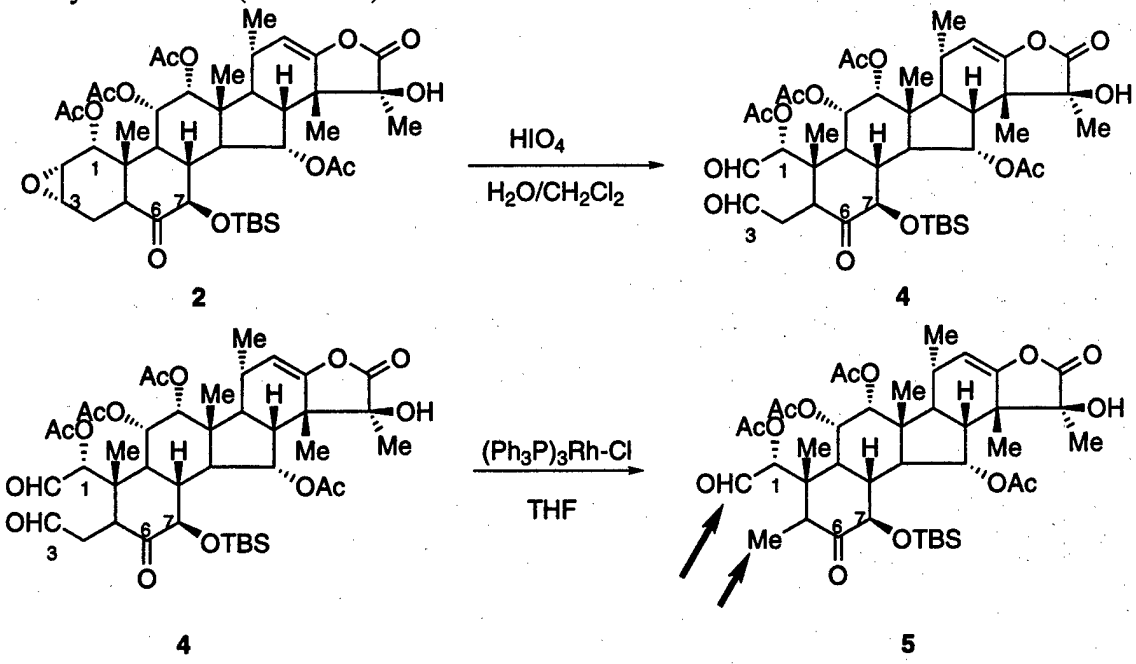
As reported in the Year 1 report, the deepoxygenation of taccalonolide at C-2/C-3 suffered from poor mass recovery at high or complete conversion of starting material. We ascribed this problem to similar polarity of the reaction product and of the reduction product(s) generated from the *bis*-cyclopentadienyl Ti^{III} reagent that is used for the deoxygenation. After considerable experimentation we have found that the isolated yield of the deoxygenation product **3** can be increased to around 65% if taccalonolide is first protected as the dimethyl *tert*.butyl silyl (TBS) ether **2** and then deoxygenated and deprotected using the triethylamine/hydrofluoric acid complex (TREAT·HF) (Scheme 1).



Scheme 1. Improved procedure for deoxygenation of taccalonolide at C-2/C-3.

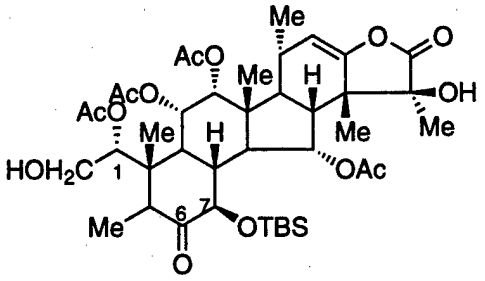
Ring cleavage of ring A

It is known from the literature that sodium periodate treatment of taccalonolide A results in the cleavage of the B-ring between the ketone at C-6 and the α–hydroxy group at C-7.[1] Provided that the α–hydroxy group is protected as the TBS ether described above, this undesired process can be suppressed and in a slow reaction periodic acid cleaves the epoxide to yield the dialdehyde **4** instead. Treatment of **4** with Wilkinson's catalyst in the presence of triethylphophorylazide[2] leads to clean loss of the aldehyde carbon atom C-3 (taccalonolide numbering) to yield **5** in an overall yield of about 65%. Through variation of the reaction conditions we have so far not been able to effect the removal of the C-2 aldehyde function (Scheme 2).

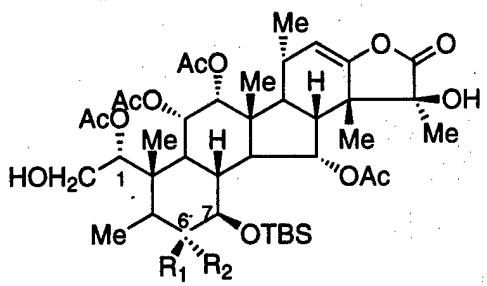


Scheme 2. Reactions leading to ring cleavage of ring A.

It is known that the method does not work if the carbon atom α to the aldehyde is disubstituted as in cyclohexane carboxaldehyde and it may be that the acetoxy function at C-1 of **5** must be regarded as such a second substituent. The failure of **5** to undergo decarbonylation to **6** was unexpected and has turned into a major roadblock. Because of the high reactivity of the aldehyde group with amino acid side chains in proteins, a masking of this group is important before this material is subjected to testing. Reduction to the alcohol can apparently not be achieved with sufficient selectivity with respect to the ketone function. Mixtures bearing alcohol functions at C-2, as desired, and/or at C-6 are invariably obtained. Although compounds **6** and **7a/b** can be accumulated in this way, the yields of pure materials are low because at C-6 stereoisomeric alcohols are being generated in the process. Moreover, the results of biological testing will not reflect the effects of selective modifications of single functional group deletions as proposed because new functional groups have been introduced in the process.



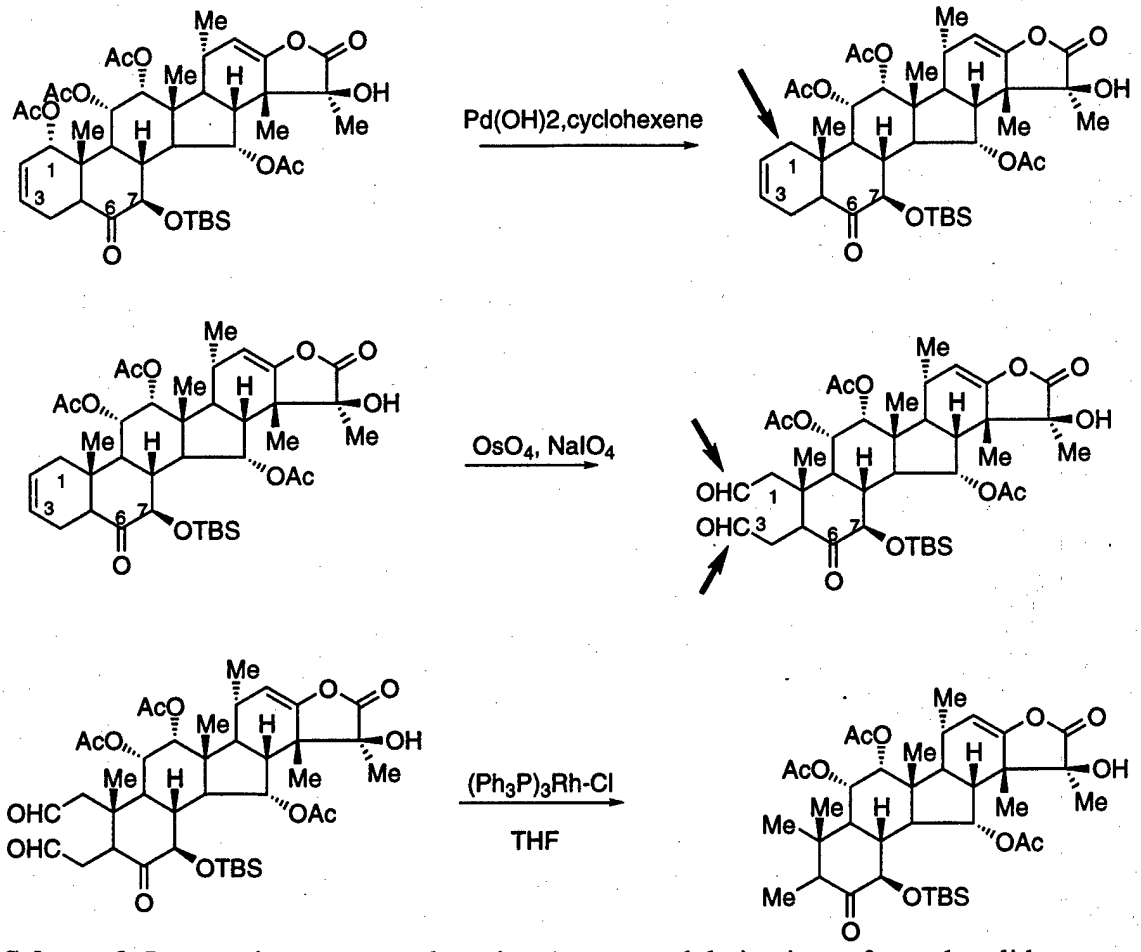
6



7a R_1= H, R_2= OH
7b R_1= OH, R_2= H

One possible solution to this problem is the conversion of the aldehyde function to an alkyne using Bestmann's reagent.[3] This carbene cannot react with ketones and should therefore be selective. The reported standard reaction conditions (K_2CO_3/methanol) need to be modified, however, because these are also standard conditions for deacetylation of acetoxy functions of which taccalonolide A bears four. The same selectivity may also be achievable using trimethysilyldiazomethane after deprotonation.[4]

However, a second and presumably better alternative is the use of the deepoxidized derivative **3** as the starting material. The acetoxy function next to the double bond in ring A is uniquely activated and can be removed by transfer hydrogenation with $Pd(OH)_2$/cyclohexene to yield **8**.[5] Literature precedent suggests that this can be done without attacking the deactivated double bond in ring E. We are presently establishing the reaction conditions on model compounds. Based on ample precedent and the successful conversion of **2** to **4**, osmium tetroxide/sodium periodate catalyzed cleavage of the double bond in **8** will yield the dialdehyde **9**. Compound **9** does not have any substituent on either of the aldehyde α carbon atoms and should be decarbonylated to the dimethyl compound (Scheme 3).



Scheme 3. Proposed new approach to ring A truncated derivatives of taccalonolide.

Task 4

As mentioned in the Year 1 report, with the departure of Dr. Mooberry from the University of Hawaii we did lose our collaborator for the cytotoxicity and antimicrotubule activity studies. Two individuals, Drs. Lorenzo and Czizar at the Cancer Center and the Pacific Biomedical Research Center (PBRC), respectively, had expressed willingness to help us out as far their own personnel situation allowed. Over the course of the past year Dr. Czizar has left PBRC and has obtained an appointment in the Medical School and had to move her laboratory. These disruptions were obviously not conducive to a start of a new collaboration. Dr. Lorenzo is still establishing her program and has not yet attracted students that could run samples for us. As a consequence of this and because of some of the preparative problems described under Task 3 we have fallen behind in our schedule.

I have approved sabbatical leave in the Spring semester of '03 starting January 1 until the end of June with pay from UH funds. I intend to use the bulk of this time to work in the laboratory myself on the goals of this award in order to get back on schedule. My efforts

will primarily focus on the chemical preparative aspects of the work plan. However, I may also learn the techniques necessary to do the biological testing.

Key Research Accomplishments

- Improved procedure for the preparation of desepoxytaccalonolide.
- Preparation of five new derivatives of taccalonolide

Reportable Outcomes

None as of yet

Conclusions

The wide variety of functional groups present within taccalonolide A makes the semisynthetic approach to structure-function analysis very challenging. While our successful improvement of the preparation of the desepoxy-derivative shows that these challenges can be met successfully, the process is slower than we had planned and hoped. Part of the problem is that for reasons of lack of suitable candidates, we are doing this work with a third year graduate student rather than a postdoc as had been the plan. At the present time I see no need to change the approach we are pursuing.

Literature cited

[1] Chen, Z.-L.; Shen, J.; Gao, Y. *Heterocycles* **1989**, *29*, 2103-2108.
[2] O'Connor, J. M.; Ma, J. *J. Org. Chem.* **1992**, *57*, 5075-5077.
[3] Müller, S.; Liepold, B.; Roth, G. J.; Bestmann, H. J. *Synlett* **1996**, 521-522.
[4] Miwa, K.; Aoyama, T.; Shiori, T. *Synlett* **1994**, 107-108.
[5] Bianco, A.; Passacantilli, P.; Righi, G. *Tetrahedron Lett.* **1989**, *30*, 1405-1408.